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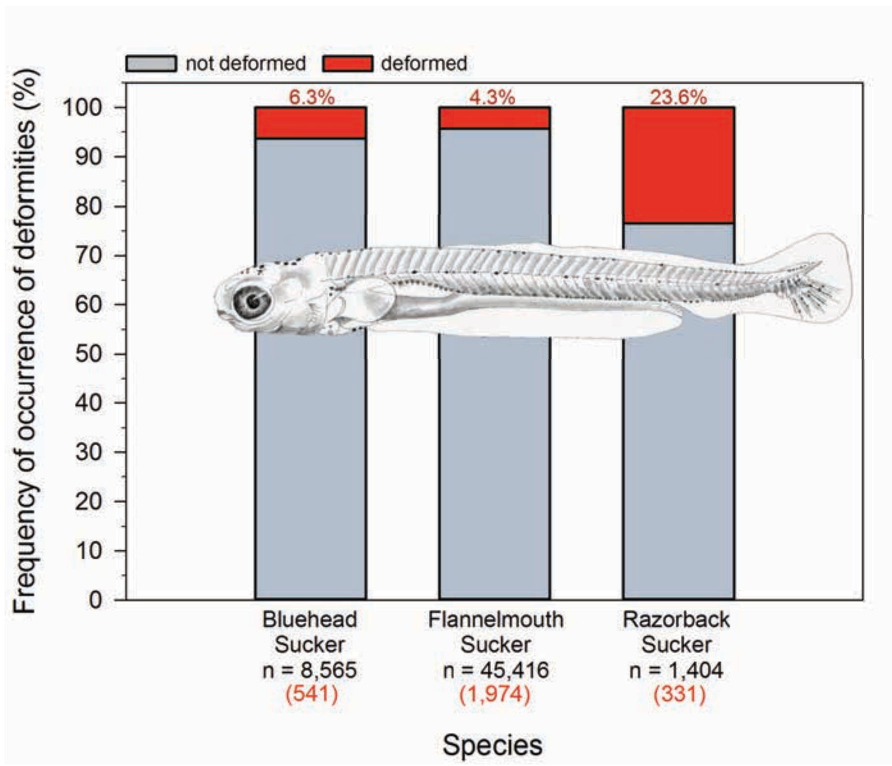
in the San Juan River from 1998 to 2012

FINAL REPORT



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San Juan River Basin Recovery Implementation Program

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Submitted to:

United States Bureau of Reclamation and the San Juan River Basin Biology Committee
under the authority of the
San Juan River Basin Recovery Implementation Program

Submitted by:

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EXECUTIVE SUMMARY

Monitoring of the San Juan River larval fish community has occurred from1998 to 2014 to help inform management decisions for federally endangered Colorado Pikeminnow, *Ptychocheilus lucius*, and Razorback Sucker, *Xyrauchen texanus*. The San Juan River population of reproducing Razorback Sucker is believed composed entirely of stocked fish. Although this species is currently maintained through augmentation efforts, reproduction has been documented annually since 1998 through the collection of wild spawned larval fishes. Recruitment of larval and early juvenile life stages of this species to the wild-spawned sub-adult or adult life stage (age-1^{+}) has not been documented but is an essential component necessary for recovery of Razorback Sucker.

Larval fish collections from 1998 to 2012 yielded observations of age-0 catostomids with opercular deformities. Opercular deformities are characterized by a shortened or curled portion of the distal gill cover resulting in exposure of gill filaments and impairment of the buccal pump system. This deformity results in increased susceptibility of fishes to gill parasites, reduced respiration and mobility, and increased mortality.

In this study, the frequency and severity of opercular deformities in Razorback Sucker was determined and compared with two sympatric native San Juan River catostomids: Bluehead Sucker, *Catostomus discobolus*, and Flannelmouth Sucker, *Catostomus latipinnis*. Catostomids were examined individually for opercular deformities (on both the left and right sides), severity of deformity rated, and specimens categorized into the larval or juvenile life stage. An information theoretic approach was used to rank candidate models using year, geomorphic reach, flow, and mesohabitat as covariates. The probability of occurrence of opercular deformities was determined annually for all three species.

To determine a baseline value of the rate of opercular deformities in brood stock of San Juan River Razorback Sucker, a sample of the 2014 Razorback Sucker propagules from Southwestern Native Aquatic Resources and Recovery Center (U.S. Fish and Wildlife Service Fish Hatchery, Dexter, NM) was retained. Most of these specimens were considerably larger (length) than wild-spawned age-0 catostomids examined for this study. Of those Razorback Sucker (n = 474) rated, 2.1% (9 fish) of fish displayed opercular deformities.

A total of 55,385 age-0 fishes were rated for opercular deformities: 8,565 Bluehead Sucker, 45,416 Flannelmouth Sucker, and 1,404 Razorback Sucker. For all years combined, frequency of opercular deformities in Razorback Sucker was 23.6%, compared with 6.3% in Bluehead Sucker, and 4.3% in Flannelmouth Sucker. Opercular deformities were primarily observed in larval fishes, versus early juvenile specimens, and deformities occurred both unilaterally and bilaterally.

Results from the modeling analysis suggest year accounts for most of the variation in the occurrence of deformities for all three species. However, flow in May and June, when larval catostomids are most abundant, best explains deformity levels in Bluehead Sucker. The other covariates (geomorphic river reach, mesohabitat, flow in May, and flow in June) collectively accounted for a negligible amount of the variation in the occurrence of deformities for all three species. Annual estimates of opercular deformities were relatively consistent across years for all three species, with the exception of a few outliers. Razorback Sucker opercular deformity level consistently occurred at higher rates across years than the other two species.

The frequency of opercular deformities was more than three times higher in Razorback Sucker than in Bluehead Sucker and more than five times higher than in Flannelmouth Sucker. In addition, opercular deformity levels in Razorback Sucker were high when compared to other studies of deformities among wild fishes in other river basins. High opercular deformity rates in Razorback Sucker larvae and juvenile in the San Juan River may be inhibiting their recruitment to later life stages.

INTRODUCTION

Major rivers in the American Southwest have been severely altered in the last century with the construction of dams and diversion of water to agriculture and urban centers. The increase of hydrologic-altering activities has led to the imperilment of many fishes within these river basins. The Colorado River Basin native fish fauna includes several endemic large-bodied, long-lived, big-river cyprinids and catostomids, many of which are federally threatened or endangered. Reduced populations of these big river fishes are attributed to the effects of dams (e.g., fragmentation, flow modifications, and cold tailwaters), channel simplification, habitat degradation, sediment input, pollutants, and interactions with non-native species (Bezzerides and Bestgen 2002, Cooke et al. 2005, Douglas and Marsh 1998, Minckley et al. 2003, Platania 1990, Ptacek et al. 2005, Rees et al. 2005, Ryden and Pfeifer 1994, Tyus and Karp 1990, Tyus and Saunders 2000).

In the San Juan River, a major Colorado River tributary, three native catostomids co-occur: Bluehead Sucker, *Catostomus discobolus,* Flannelmouth Sucker, *Catostomus latipinnis*, and federally endangered Razorback Sucker, *Xyrauchen texanus* (U.S. Fish and Wildlife Service 1991). Bluehead Sucker and Flannelmouth Sucker are considered sensitive species throughout their ranges (Rees 2005); however, populations of these species in the San Juan River are relatively stable (Ptacek et al. 2005, Rees et al. 2005, Schleicher and Ryden 2012). Federal listing of Razorback Sucker resulted in recovery efforts, including stocking of hatchery-reared adult Razorback Sucker into the San Juan River beginning in 1997 (Ryden 1997, U.S. Fish and Wildlife Service 1998). The San Juan River population of reproducing Razorback Sucker is believed composed entirely of stocked fish. Although this species is currently maintained through augmentation efforts (Furr 2013, Schleicher 2013), and reproduction has been documented through the annual collection of larval fishes (Farrington et al. 2013), recruitment of larval and early juveniles of this species to the sub-adult or adult life stage (age-1^+) has not yet been documented. Recruitment failure over the past two decades has seriously inhibited the successful recovery of Razorback Sucker in the San Juan River.

Monitoring of the San Juan River larval fish community, performed to help inform management decisions, has been conducted as part of the San Juan River Basin Recovery Implementation Program (SJRBRIP) since 1998 (San Juan River Basin Recovery Implementation Program 1995, San Juan River Basin Recovery Implementation Program 2013). Reproduction by stocked Razorback Sucker was first discovered in the San Juan River in 1998 (Brandenburg 2000). The San Juan River Larval Fish Monitoring Project has, in addition to documenting 16 consecutive years of spawning by Razorback Sucker, demonstrated an increase in the reproductive output of the population and an upstream increase in the range of spawning individuals (Farrington et al. 2013). Understanding important aspects of larval fish ecology such as survivorship, developmental rates, rearing habitat and water quality requirements, and larval fish community composition can benefit efforts to decrease mortality of native larval fishes and increase recruitment to the reproductive developmental stage.

Mortality rates are high in the early ontogeny of fishes (Harvey 1991, Jennings and Philipp 1994) and may be attributed to abiotic and/or biotic factors. Environmental variables, such as unsuitable rearing habitat, unfavorable water temperatures, and low dissolved oxygen, reduce survivorship of larval fishes. Competition for food resources and predation also impact survivorship, and are intensified by the introduction of non-native fishes (Carpenter and Mueller 2008). Additionally, many larval fishes absorb their yolk before finding adequate habitat with appropriate food resources and starve (reviewed in Miller and Kendall 2009).

In addition to the suite of factors affecting the recruitment of larval fishes to the adult life stage, deformed opercula have been observed in age-0 catostomids collected in the San Juan River during larval fish monitoring. The opercular bone has an anterior articulation with the hyomandibula and covers and protects the gills of juvenile and adult fishes. During early life stages of San Juan River catostomids, the operculum is initially a soft and flexible tissue that covers the gills before it ossifies about 30 – 50 days after hatching.

Opercular deformities occur when the distal (i.e., away from the point of attachment) portion of the gill cover is shortened or curled, exposing gills and impairing the buccal pump system used for feeding and breathing (Beraldo et al. 2003, Boglione et al. 2013b, Koumoundouros et al. 1997). Opercular deformities develop during the pre-flexion and flexion stages of development (Koumoundouros et al. 2010). Opercular deformities, which occur across fish taxa, may inhibit growth, function (e.g., feeding and mobility), increase gill disease, and result in mortality (Abdel et al. 2004, Beraldo et al. 2003, Koumoundouros et al. 1997, Paperna et al. 1980). Malformed opercula can occur on both sides of an individual fish but are more commonly observed unilaterally (e.g., Abdel et al. 2004, Al-Harbi 2001, Beraldo 2003, Koumoundouros et al. 1997, Verghaegen et al. 2007). Many cultured species exhibit malformed opercula (Al-Harbi 2001, Fraser and de Nys 2005, Gapasin et al. 1998, and reviewed in Boglione 2013b); however, few studies have described the frequency of occurrence of opercular deformities in wild fish populations (Lindesjoo et al. 1994, Plunkett and Snyder-Conn 2000, Sun et al. 2009).

As part of the San Juan River Larval Fish Monitoring Project, a high number of opercular deformities were incidentally observed in larval Razorback Sucker during laboratory identification of specimens in 2011. This discovery was reported to the SJRBRIP at the February and May 2012 meetings. Because of the possible lethal nature of this deformity, the SJRBRIP requested a study on the temporal and spatial extent of opercular deformities in larval and juvenile Razorback Sucker in the San Juan River.

This study determined the frequency of occurrence and relative severity of opercular deformities in age-0 Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker collected in the San Juan River from 1998 to 2012. Data were analyzed to determine the occurrence of opercular deformities for the three native catostomids, determine the severity of deformities by developmental stage (larval and juvenile), and describe the side of the fish affected by deformities. An information theoretic approach was used to rank models of variables potentially affecting the occurrence of opercular deformities, and the estimated probability of occurrence of deformities for all three species by year was determined. Study objectives were derived from Task 4.1.6.2 in the San Juan River Long Range Plan.

OBJECTIVES

LRP Task 4.1.6.2 Investigate potential health problems, identify causes, and recommend corrective actions if any indications of poor health are of concern.

The work proposed will be conducted under the SJRBRIP. The objectives of this specific project are identified and listed below.

1) Examine catostomid specimens collected from 1998 to 2012 for opercular shortening from field-collected specimens obtained from the San Juan River Larval Fish Monitoring Project.

2) Record species and severity of opercular deformities bilaterally on a scale of 0 (none), 1 (slight shortening), or 2 (severe shortening).

3) Record developmental stage of each specimen as either larval or juvenile.

4) Analyze spatiotemporal relationship of opercular deformities by severity, species, developmental stage, side affected, hydrologic regime, and water temperature.

STUDY AREA

The San Juan River is a 224-mile-long segment extending from Navajo Dam to Lake Powell. The river originates in the San Juan Mountains of Colorado and flows through New Mexico, Colorado, and Utah. Several major perennial tributaries flow into the San Juan River. Additionally, there are numerous ephemeral arroyos that contribute relatively little flow annually but input large sediment loads during rain events. Historically, the river hydrology had high spring flows driven by snowmelt runoff, followed by low flows, with summer and fall monsoon flow spike events. In 1963, Navajo Dam and Navajo Reservoir became operational, and the resulting water operations have been partially managed to recreate natural flow regimes since 1992 (Thomas et al. 1998). The San Juan River Flow Recommendations (Holden 1999), created within the SJRBRIP, were designed to maintain spawning and migration cues, suppress non-native fish populations, and create nursery habitat for larval fishes.

The principal study area for fisheries investigations in the San Juan River is the 180-mile reach between Farmington and Lake Powell. This segment comprises six geomorphic river reaches (Bliesner and Lamarra 1999; Figure 1) with Reach 6 being the most upstream reach of the study area, and Reach 1 ending at Lake Powell. All samples examined for this study were taken during San Juan River Larval Fish Monitoring and were collected in Reaches 5 to 1.

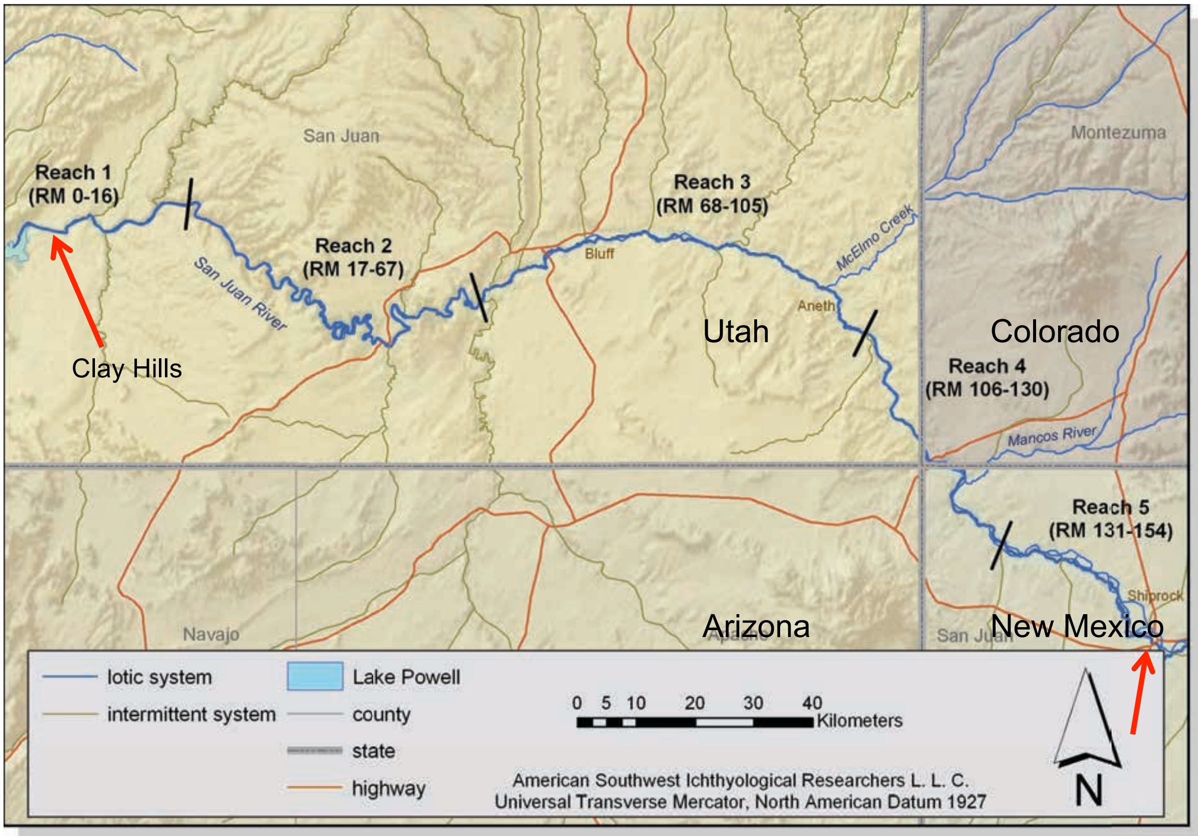

Figure 1. San Juan River study area from Shiprock, NM (red arrow, lower right) to Clay Hills, UT (red arrow, upper left; near Lake Powell inlet).

METHODS

Specimens used in this study were collected during 1998 – 2012 larval fish monitoring surveys conducted on the San Juan River. The study area for the Larval Fish Monitoring Project has remained relatively constant since 1999 (Table 1). The post-1999 increase in upstream range of larval fish sampling was the result of the expanded range of reproducing Razorback Sucker. The original sampling period of the San Juan River Larval Fish Monitoring Project (April through June) was designed to maximize collection of larval catostomids and was subsequently expanded in 2002 to allow for the collection of larval cyprinids (July – September). In 2011, the sampling regime was again modified and September sampling eliminated due to the lack of larval fish present at that time of year. An important and consistent aspect of the San Juan River Larval Fish Monitoring Project has been extensive annual sampling during the period that larval catostomids are present in the system (April – June).

Access to the river and collection localities was gained through the use of 16' inflatable rafts. Sampling of the entire study area was accomplished during a one week period. There was not a predetermined number of collections per river mile or geomorphic reach. Instead, collections were made in as many suitable larval fish habitats as possible within the river reach being sampled. Previous San Juan River investigations clearly demonstrated that larval fish most frequently occur and are most abundant in low velocity habitats such as pools and backwaters (Lashmett 1993). Sampling of the entire study area was accomplished during a one week period (Farrington et al. 2013).

Collecting sites for the Larval Fish Monitoring Project were chosen based on presence of suitable low velocity aquatic habitats. The primary mesohabitats sampled were backwaters, embayments, pools, isolated pools, and slack waters. Specimens were collected using small mesh seines (1 m x 1 m x 0.8 mm mesh). Several seine hauls (between two and six) were made through an individual mesohabitat depending on the size of that habitat. Those fishes too small to identify in the field were preserved in 5% formalin (1998 – 2008) or 95% ethyl alcohol (2009 – 2012). Samples were returned to the laboratory where they were sorted and identified to species. Specimens were identified by personnel with expertise in San Juan River Basin larval fish identification. Stereo-microscopes with transmitted light bases and polarized light filters were used to aid in identification of larval individuals. Age-0 specimens were separated from age-1^{+} specimens using published literature that define growth and development rates for individual species (Auer 1982, Snyder 1981, Snyder and Muth 2004). Both age classes were enumerated and cataloged in the Museum of Southwestern Biology (MSB), Division of Fishes at the University of New Mexico (UNM) (Farrington et al. 2013).

All collections from 1998 to 2012 that contained native catostomids (Bluehead Sucker, Flannelmouth Sucker, and Razorback Sucker) were examined for frequency and severity of opercular deformities (Figure 2). The opercula are not fully developed until at least the post-flexion mesolarval stage of development. Because of varying lengths at developmental stages for different species, a species-specific minimum body size (total length = TL) was determined to ensure fully developed opercula: 16 mm TL for Bluehead Sucker, 19 mm TL for Flannelmouth Sucker, and 15 mm TL for Razorback Sucker. Age-0 specimens meeting the minimum length requirements were examined individually for opercular deformities on both left and right sides following Abdel et al. (2004) (Figure 3).

In addition to the presence/absence rating scale used in other opercular deformity studies (Koumoundouros et al. 1997, Gapasin et al. 1998, Gapasin and Duray 2001, Sun et al. 2009), this study categorized the severity of shortening. Severity of shortening was assessed and rated as level 0 (no opercular deformity), level 1 (slight shortening), or level 2 (severe shortening) (Figure 4). Slight shortening (level 1) ranged from separation of the operculum from near the origin of the pectoral fin to the first row of gill filaments. Severe shortening (level 2) was determined by the exposure of more than one row of gill filaments. For fish with opercular deformities (level 1 or level 2) total length was recorded.

Age-0 specimens meeting the minimum size requirements were categorized by developmental stage as either larval or juvenile. Larval fish is a specific developmental period between the time of hatching and when individuals transform to the juvenile stage. Juvenile fishes are those that no longer retain traits characteristic of larval fishes and are differentiated from larvae by the complete absorption of their fin folds and full complement of median and paired fin rays (Snyder and Muth 2004).

OPERCULAR DEFORMITIES IN HATCHERY STOCK RAZORBACK SUCKER

The source of nearly all Razorback Sucker stocked in the San Juan River is the U.S. Fish and Wildlife Service's Southwestern Native Aquatic Resources and Recovery Center (SNARC), Dexter, NM (formerly Dexter National Fish Hatchery and Technology Center). In February 2014, after presentation of the initial results of this study, the SJRBRIP Biology Committee requested information on the rate of opercular deformities in larval and early juvenile Razorback Sucker spawned at SNARC (prior to release). The goal of this preliminary investigation was to determine if an underlying genetic component could explain the high frequency of opercular deformities in wild age-0 Razorback Sucker, the presumed progeny of adult hatchery-released fish.

To determine a baseline rate of opercular deformities in San Juan River Razorback Sucker broodstock, a sample of the 2014 Razorback Sucker propagules was retained. Adult Razorback Sucker were spawned on 1 April 2014 and the sample of propagules (n = 474) was preserved in 10% formalin on 29 May 2014. Specimens meeting the size requirements were examined individually for opercular deformities on the left and right sides and the severity of the deformities was categorized as level 0, 1, or 2. Developmental stage, larval or juvenile, was determined for each specimen.

To initially determine the prevalence of opercular deformities in hatchery stock of Razorback Sucker, the frequency of deformities was calculated for larval and juvenile life stages. Further analysis was not possible because the low number of deformities violated the assumptions of the Chi-square test.

Table 1. Years, months, and reaches sampled for larval fishes in the San Juan River from 1998 to 2012.

Years	Reaches	Months Sampled
1998	4,3,2	April – June
1999	4,3,2,1	April – June
2000	4,3,2,1	April – June
2001	5,4,3,2,1	April – June
2002 – 2011	5,4,3,2,1	April – September
2012	5,4,3,2,1	April – August

Figure 2. Opercular deformities in age-0 catostomids from the San Juan River. The range of severity for deformities is shown, with decreasing severity from top to bottom. Differences in deformity severity could translate to differential survivorship or impairment, therefore a categorical rating scale was used.

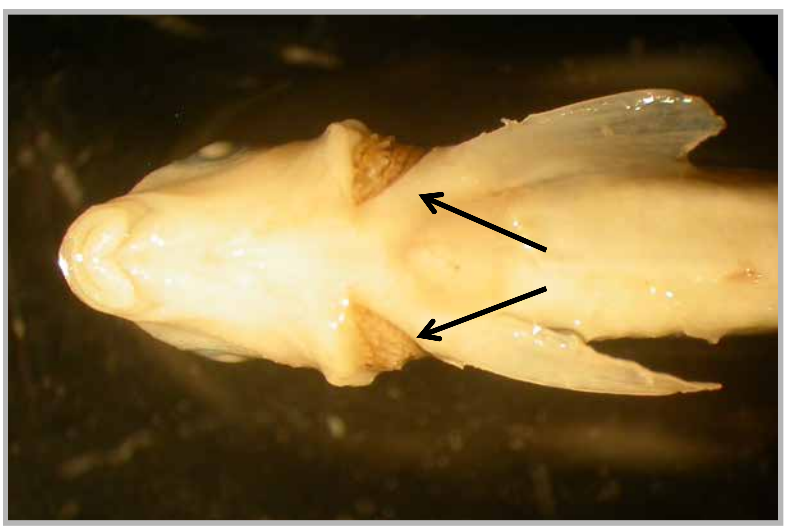

Figure 3. Severely deformed opercula (level 2), displayed bilaterally, in a ventral view of an age-0 Flannelmouth Sucker from the San Juan River. Opercular flaps are shortened and curled, exposing several rows of gill filaments.

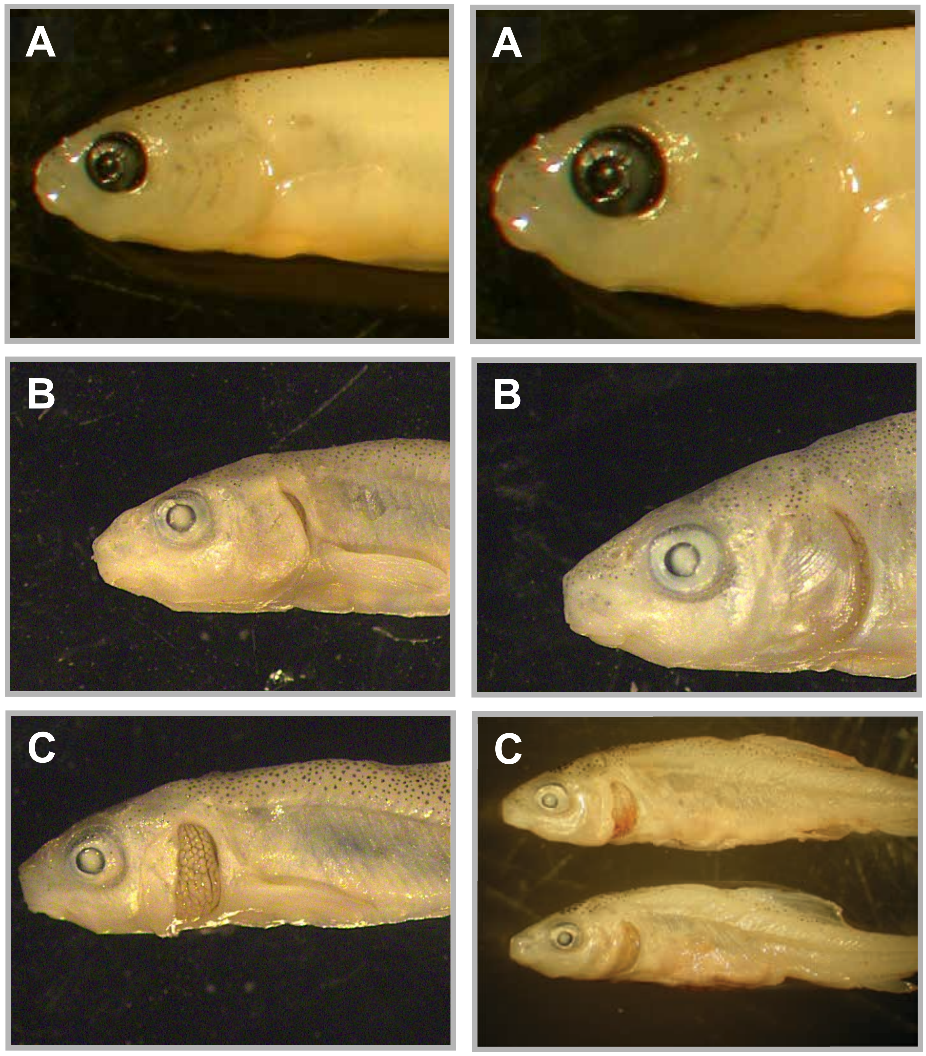

Figure 4. Scale used for rating opercular deformities in age-0 catostomids. The top row (A) of a catostomid without an opercular deformity (level 0), middle row (B) of catostomids displaying slight shortening of operculum (level 1), and the bottom row of catostomids (C) displaying severely deformed opercula (level 2).

ANALYSES

Data were tested for differences in occurrence of opercular deformities among all species from 1998 to 2012, using the presence/absence of deformities. For each species, the frequency and severity of deformities found in larvae or juveniles were examined. Additionally, we described whether deformities were bilateral or unilateral, and whether deformities were more common on the right or left sides. These analyses were performed using contingency table data and the chi-square test (JMP 2007). The null hypothesis of no differences in frequencies of opercular deformities across categories was rejected at $P < 0.05$.

Variables potentially affecting the occurrence of opercular deformities were also examined. For each species, an information theoretic approach was applied to rank candidate models using year, geomorphic reach, flow, and mesohabitat. The Akaike Information Criterion (Akaike 1973), corrected for small sample sizes (AIC_C), was used to rank multiple logistic regression models constructed with environmental covariates potentially affecting the occurrence of opercular deformities in age-0 catostomids. Juvenile fishes were not included in the models because of their low representation in collections. Because water temperature was auto-correlated with flow, and temperatures recorded in the main channel were not representative of larval fish habitat, discharge measurements were used in the model (USGS gage station 09379500 near Bluff, UT). Flow attributes incorporated in the model were the average monthly mean daily discharge (Q) in May and June, the period when larval catostomids were most abundant in samples. Seven competing models were analyzed, which included six covariate models and a single null model that assumed no difference in the estimated probability of deformities across years.

The estimated probability of occurrence of opercular deformities in age-0 catostomids was determined for each of the 16 years of the study and for the three species. Deformity data were analyzed using a multiple logistic regression model in PROC NLMIXED (SAS 2014), a numerical optimization procedure. Logistic regression was used to model the probability that a sampling site in the study area contained larval catostomids with an opercular deformity. These models provided estimates of the probability of occurrence of deformities (p) and the standard deviation of the normal distribution around that value. Sigma added extra-binomial variation, on the logit scale, to the logistic regression.

The relative fit of the data to various models was assessed using goodness-of-fit statistics (*log*Like = -2[*log*-likelihood]) and AIC_C (Akaike 1973, Burnham and Anderson 2002). Lower values of AIC_C indicate a better fit of the model to the data. Models were ranked by AIC_C values and included AIC_C weight (w_i) and ΔAIC.

RESULTS

From the 1998 to 2012 collections, 55,385 catostomids were rated for opercular deformities. Flannelmouth Sucker represented 82.0% (n = 45,416) of the total specimens examined, followed by Bluehead Sucker at 15.5% (n = 8,565), and Razorback Sucker at 2.5% (n = 1,404).

INTERSPECIES VARIATION

The frequency of individuals with and without deformities differed significantly among species (n = 55,385, $\chi^2 = 1{,}061.9$, $P < 0.001$), with a higher frequency of opercular deformities in Razorback Sucker (23.6%), and similar frequencies recorded in Bluehead Sucker (6.3%) and Flannelmouth Sucker (4.3%) from 1998 to 2012 (Figure 5).

SEVERITY OF DEFORMITIES BY LIFE STAGE

For all three species, most fish rated were in the larval life stage (Bluehead Sucker, 74%; Flannelmouth Sucker, 68%; Razorback Sucker, 90%) and more deformities occurred in the larval life stage (Bluehead Sucker, 6.8%; Flannelmouth Sucker, 5.7%; Razorback Sucker, 25.9%) than in the juvenile life stage (Bluehead Sucker, 5.0%; Flannelmouth Sucker, 1.5%; Razorback Sucker, 1.5%). A higher frequency of severe deformities occurred in the juvenile stage, compared to larval stage, for Bluehead Sucker (n = 541, $\chi^2 = 51.7$, $P < 0.001$) and Flannelmouth Sucker (n = 1,974, $\chi^2 = 50.7$, $P < 0.001$; Figure 6). A chi-square test was not used with Razorback Sucker because the low sample size of juveniles (n = 2, both with severe deformities) violated assumptions of the test.

SIDE DEFORMED

For Bluehead Sucker and Flannelmouth Sucker, most deformities displayed unilaterally. The side (left, right, both) where deformities occurred was significantly different in Bluehead Sucker (n = 25,695, $\chi^2 = 27.834$, $P < 0.001$), with 61% of deformities unilateral (Left: 62%, Right: 38%). Similarly, the side where deformities occurred differed significantly in Flannelmouth Sucker (n = 136,248, $\chi^2 = 56.017$, $P < 0.001$), with the majority (59%) of deformities displaying unilaterally (Left: 52%, Right: 48%). In Razorback Sucker, the side (left, right, both) where deformities occurred varied significantly (n = 4,212, $\chi^2 = 61.695$, $P < 0.001$). Unilateral and bilateral deformities in Razorback Sucker were equally distributed (unilateral: 50%, bilateral: 50%) and most unilateral deformities occurred on the left side (Left: 67%, Right: 33%).

MODEL SELECTION

Of the seven candidate models created to explain the occurrence of deformities for each species, the model containing only year received substantial weight (w_i) for Bluehead Sucker (40%), Flannelmouth Sucker (100%), and Razorback Sucker (44%) (Table 2). The model including May and June Q received higher support ($w_i = 56\%$) for Bluehead Sucker. The null model received substantial support ($w_i = 28\%$) for Razorback Sucker but not for either Bluehead Sucker or Flannelmouth Sucker. Models that included other covariates (e.g., geomorphic reach, and mesohabitat) had low weights (i.e., low support) within the AIC_C rankings for all three species.

The probability of deformity for each year was computed as part of a binomial model for all three species (Figures 7 and 8). Estimated opercular deformity levels for Razorback Sucker were higher almost every year (range 0-100%) than the estimated opercular deformity level for Bluehead Sucker (range 0-17.7%) or Flannelmouth Sucker (range 0.8-12.9%). For all three species, the probability of occurrence of opercular deformities is relatively stable across years, with 95% confidence intervals overlapping in most years. Differences across years are attributed to low probability of deformities in 2006. In 2008, all Razorback Sucker examined had opercular deformities, but only six specimens met the size criteria necessary to be rated. Prior to 2006, annual Razorback Sucker captures were rare (n

< 200; Farrington et al. 2013), with the exception of 2002 (n = 818 captured, 403 fish rated) and 2003 (n = 472 captures, 169 fish rated).

OPERCULAR DEFORMITIES IN HATCHERY STOCK RAZORBACK SUCKER

In the Southwestern Native Aquatic Resources and Recovery Center sample of Razorback Sucker, individuals (n = 474) ranged in size from 17– 31 mm SL with 89% (51 fish) in the metalarval stage. Almost all of the specimens rated for deformities were considerably larger (length) than wild-spawned age-0 catostomids examined for the 1998 to 2012 opercular deformity study. Opercular deformities were present at a low level, 2.1% (9 fish). All opercular deformities were unilateral and rated as low severity (level 1) with most of those individuals (6 fish) being in the larval developmental stage.

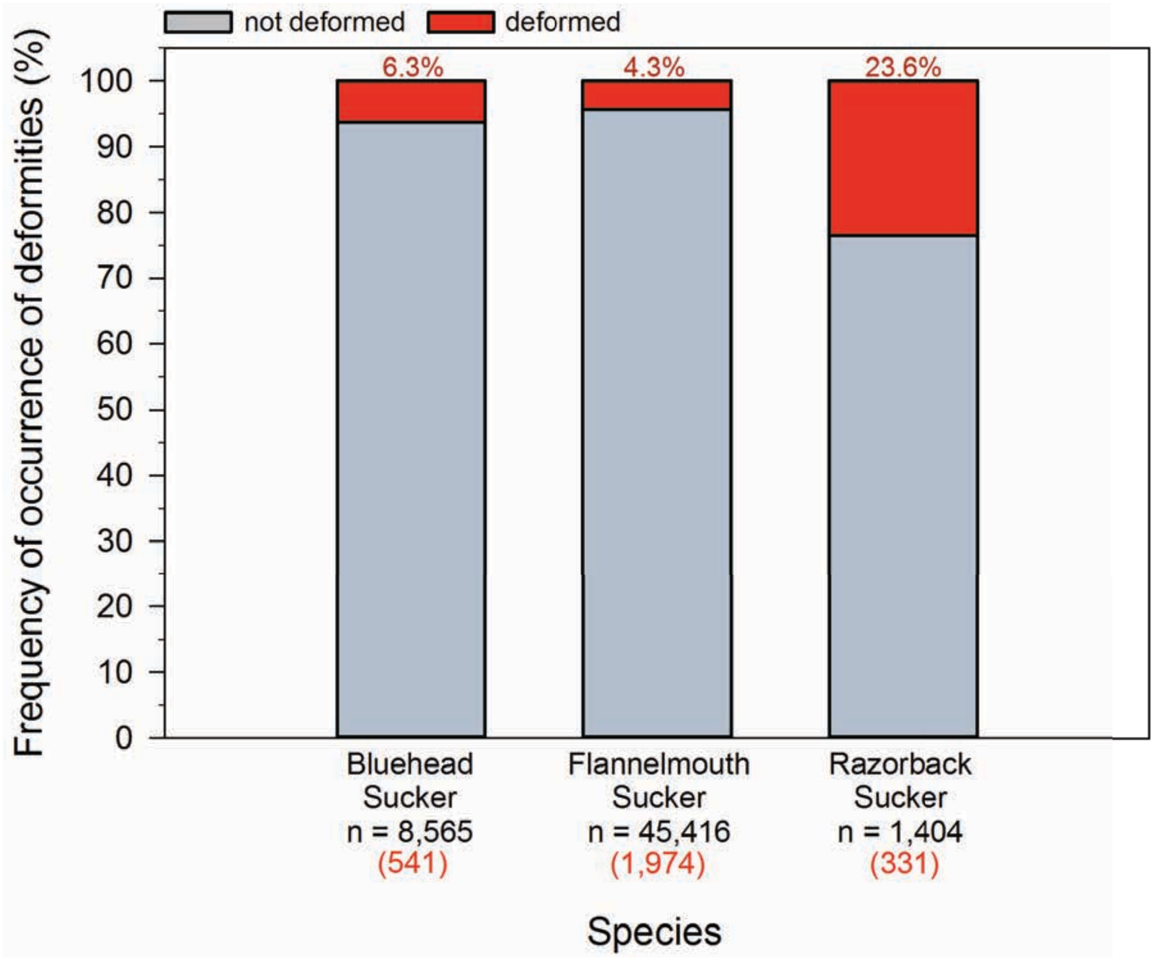

Figure 5. Frequency of occurrence of opercular deformities from 1998 to 2012 for each species. Numbers in black text are total specimens rated for each species life stage and red text in parentheses are the number with opercular deformities. Numbers above the bars are the percent of individuals with deformities.

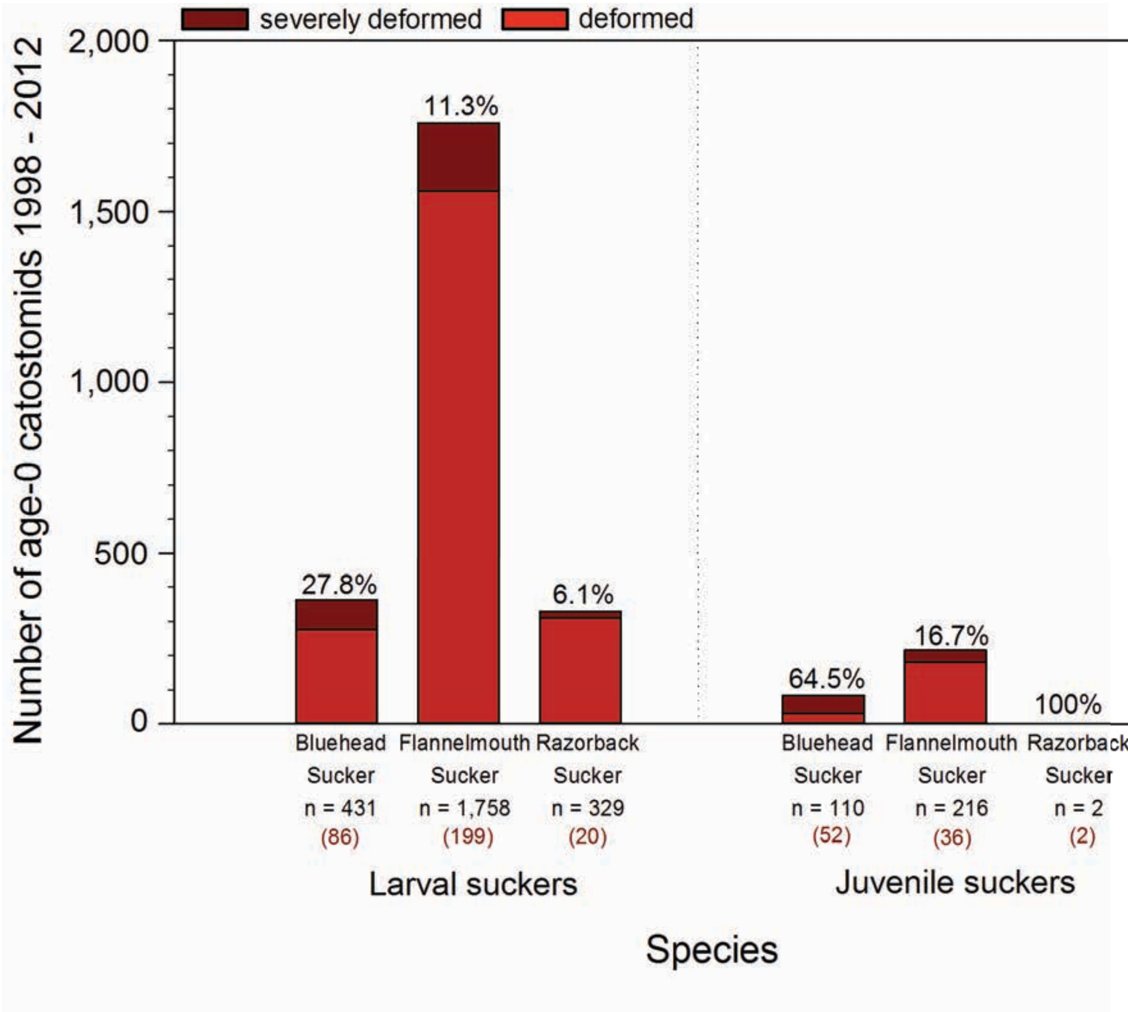

Figure 6. Number of deformities (level 1 and level 2) and number of severe deformities (level 2) for each life-stage of age-0 San Juan River catostomids from 1998 to 2012. Numbers in black text are total specimens rated for each species in each life stage and dark red text in parentheses are the number with opercular deformities. Numbers above the bars are the percent of individuals with severe deformities.

Table 2. Multiple logistic models of the occurrence of opercular deformities in age-0 Bluehead Sucker (top), Flannelmouth Sucker (middle), and Razorback Sucker (bottom), using long-term data (1998 – 2012) and spatial covariates. Models were ranked using AIC_c and included the number of parameters (*K*) and the model weight (w_i).

Model	***K***	**Log-likelihood**	**AIC_C**	**ΔAIC_C**	**w_i**
		Bluehead Sucker			
May *Q* + June *Q*	4	590.3	598.5	0	0.555
Year	13	571.5	599.1	0.6	0.403
Habitat	11	581.8	605.0	6.5	0.021
May *Q*	3	600.0	606.1	7.6	0.012
June *Q*	3	601.8	608.0	9.5	0.005
Null	2	605.0	609.1	10.6	0.003
Reach	7	597.7	612.2	13.7	0.001
		Flannelmouth Sucker			
Year	16	2380.8	2413.5	0	1.000
May *Q* + June *Q*	4	2454.4	2462.5	48.9	0.000
Habitat	11	2446.4	2468.7	55.2	0.000
June *Q*	3	2463.2	2469.2	55.7	0.000
Reach	7	2455.1	2469.2	55.7	0.000
May *Q*	3	2491.0	2497.0	83.5	0.000
Null	2	2496.6	2500.6	87.1	0.000
		Razorback Sucker			
Year	15	311.8	346.6	0	0.444
Null	2	343.4	347.5	48.9	0.282
June *Q*	3	343.1	349.4	55.2	0.110
May *Q*	3	343.3	349.5	55.7	0.104
May *Q* + June *Q*	4	343.1	351.5	55.7	0.038
Reach	6	340.0	352.7	83.5	0.020
Habitat	8	340.1	357.5	87.1	0.002

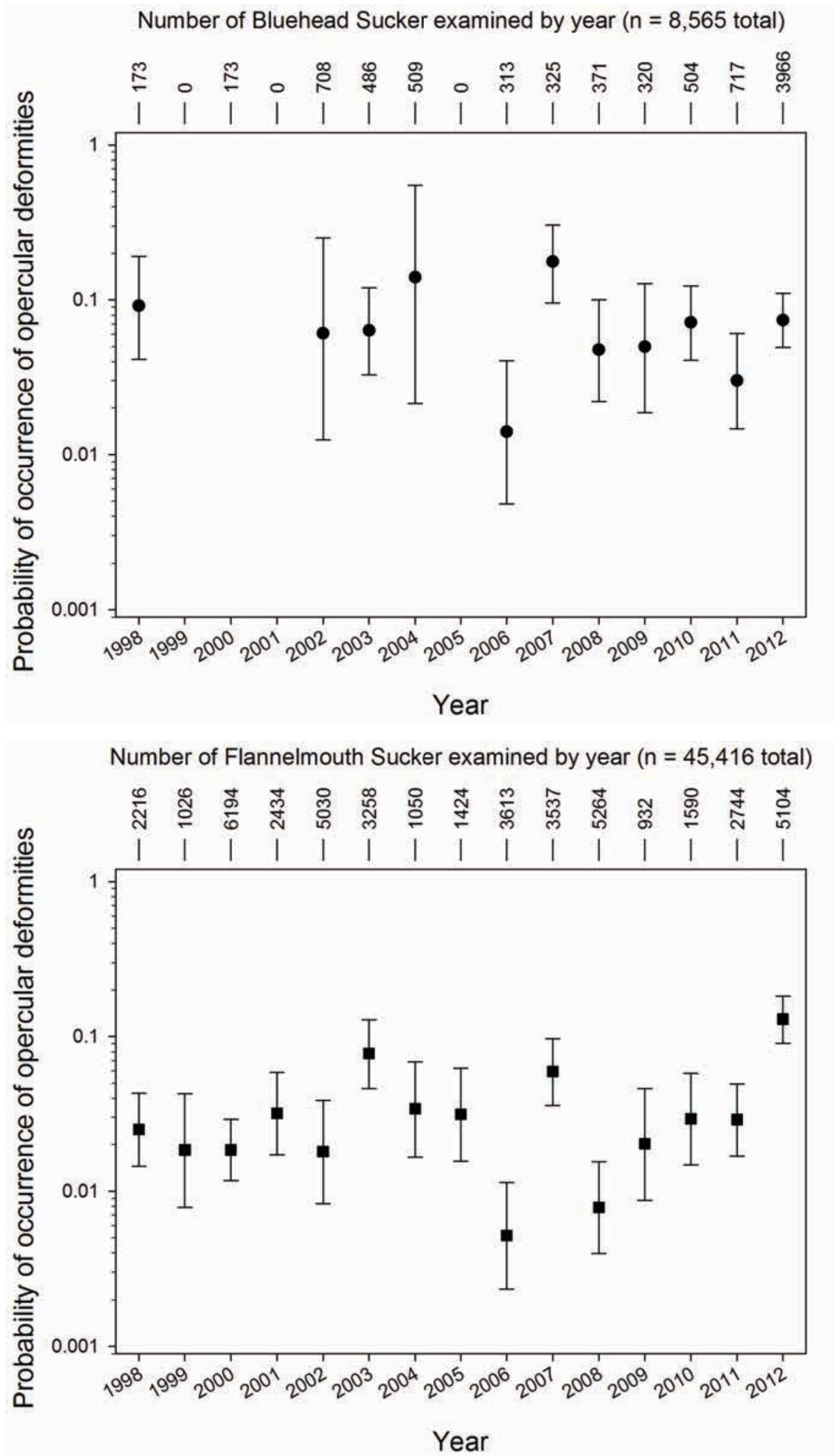

Figure 7. Estimated occurrence of deformities by year for Bluehead Sucker (top) and Flannelmouth Sucker (bottom). The number of specimens examined per year is presented above each upper X axis. Error bars represent 95% confidence intervals.

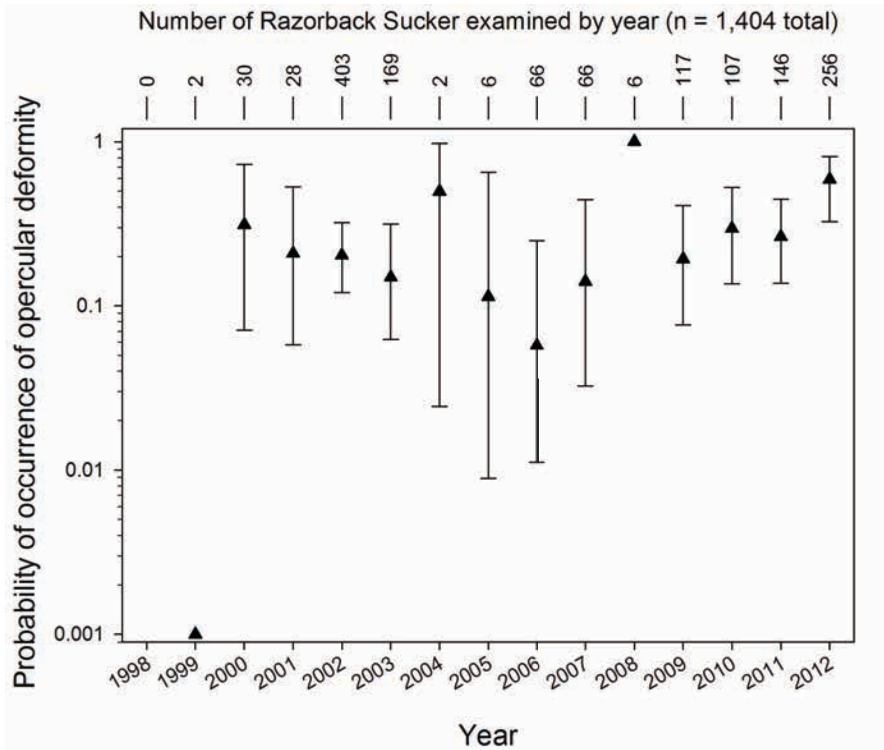

Figure 8. Estimated occurrence of deformities by year for Razorback Sucker. The number of specimens examined per year is presented above the upper X axis. Error bars represent 95% confidence intervals.

DISCUSSION

Opercular deformities were found in all three age-0 native catostomids in the San Juan River based on data collected from 1998 to 2012, and were relatively high in Razorback Sucker (23.6%) as compared to Bluehead Sucker and Flannelmouth Sucker (4.3% and 6.3%, respectively). Typical background levels of skeletal anomalies in wild fishes are 1 – 3% (Lemly 2002, Lindesjoo et al. 1994, Sun et al. 2009, Patten 1968, Plunkett and Snyder-Conn 2000), indicating that opercular deformities are abnormally high in endangered Razorback Sucker, and also somewhat elevated in Bluehead and Flannelmouth Suckers. This is one of only a few studies describing opercular anomalies in wild fishes.

Age-0 catostomids in the larval life stage exhibited more deformities than juveniles, suggesting that the deformity is either lethal larval fishes or that deformed individuals are recovering (i.e., regenerating the operculum). Additionally, adult Bluehead Sucker and Flannelmouth Sucker in the San Juan River are typically not observed with deformed opercula (Jason Davis, U.S. Fish and Wildlife Service, pers. comm., Schleicher and Ryder 2012). Opercular deformities are likely lethal to younger life stages but may also contribute to reduced survivorship for juvenile and adult life stages (Boglione et al. 2013a, Lemly 1997a). If opercular deformities do not result in mortality, the fish may have reduced performance because of the impairment to the buccal-opercula pump system, limiting oxygen uptake rates and feeding ability, or exposing gills to parasites. Partial recovery has been shown in juvenile Gilthead Sea Bream with malformed opercula but only when the deformity was minor (Beraldo and Canavese 2011). In this study, more severely deformed opercula occur in a greater percentage of juvenile fishes, suggesting that if the fish survives the earliest life stages, the deformity may intensify or the operculum may not continue to grow as the fish ages. However, relatively few age-0 juvenile fishes were available to be rated for deformities. The high percentage of juveniles with severe deformities, likely resulting in mortality, and the rare observations of adults with opercular deformities suggests that catostomids with opercular deformities likely do not reach sub-adult or adult life stages.

Opercular deformities were observed bilaterally and unilaterally on all three species but were found more commonly on the left side, especially in Razorback Sucker. Deformities displaying side-independence (i.e., fluctuating asymmetry) may indicate an environmental origin because environmental factors are thought to affect both sides of the fish equally (Barahona-Fernandes 1982, Koumoundouros et al. 1997, Galeotti et al. 2000). In contrast, directional asymmetry (i.e., deformities more common on one side) may have an underlying genetic component (Fernandez et al. 2008, Verhaegen et al. 2007). Directional asymmetry occurs in inbred cichlids (Winemiller and Tayler 1982), but other studies are contradictory about linking relatedness to deformities (Tave and Handwerker 1994, Verhaegen et al. 2007) and suggest a gene X environment interaction (Boglione et al. 2013b, Kause et al. 2007). Reduced genetic variability in Razorback Sucker in the Upper Colorado River Basin, evidenced by high levels of relatedness (Dowling et al. 2012), may predispose larval fishes to opercular deformities from exposure to environmental variables.

Year explains the occurrence of opercular deformities for all species; however, variation is notable and remarkably similar across years. In general, the differences across years are driven by a few outliers, particularly for Razorback Sucker. Flow in May and June explains deformities in Bluehead Sucker, and although flow is variable across years, deformities are consistently low. In general, no clear environmental variable explains the occurrence of deformities in larval catostomids. The relatively consistent occurrence of deformities across years may indicate that environmental conditions contributing to opercular deformities remain constant as well.

Razorback Sucker display more than three times the frequency of opercular deformities than Bluehead Sucker or Flannelmouth Sucker. Razorback Sucker may be inherently more susceptible to defomities, or high levels of relatedness (Dowling et al. 2012) may contribute to higher deformities when exposed to certain environmental conditions. Across watersheds, opercular deformities are not limited to one taxon (Lemly 1997a, Lindesjoo et al. 1994, Patten 1968, Plunkett and Snyder-Conn 2000, Sun et al. 2009), but in the San Juan River, opercular deformities are not prevalent outside of age-0 catostomids. Many catostomids in western rivers are sensitive species (Bezzerides and Bestgen 2002, Colorado River Fish and Wildlife Council 2004, Cook et al. 2005, Ptacek et al. 2005, Rees et al. 2005). As benthic feeders in the detrital food web, adult catostomids are exposed to contaminants that accumulate in sediments which may be transferred to larvae. Additionally, larval fishes are found in habitats with low to zero velocity currents (such as backwaters) where environmental factors may be more pronounced because of water depth (affecting temperature and productivity) and retention times.

The level of opercular deformities in Razorback Sucker was high, especially compared to others studies of wild fishes where high frequencies of deformities were usually associated with environmental contaminants or poor water quality (e.g., Lindesjoo et al. 1994, Plunkett and Snyder-Conn 2000, Sun et al. 2009). European Perch, *Perca fluviatilis,* in brackish estuaries affected by pulp mill effluents display as much as 20% opercular deformities, compared with 1.4% in unaffected areas (Lindesjoo et al. 1994). Tilapia (*Oreochromis* spp.) in polluted waters in Taiwan had deformed opercula frequencies that ranged from 0% in the dry season to 14% in the rainy season (Sun et al. 2009). In catostomids, opercular deformities were found in larval and juvenile Lost River Sucker, *Deltistes luxatus* (4.7%) and Shortnose Sucker, *Chasmistes brevirostris,* (2.7%) associated with poor water quality in Upper Klamath Lake, Oregon (Plunkett and Snyder-Conn 2000). Deformities in wild fishes are less prevalent than in cultured fishes, where opercular deformities can affect as much as 80% of individuals across many species (described in Koumoundouros et al. 1997, Boglione et al. 2013b). In fish culture, deformities are displayed in early life stages, and deformity rates have been reduced by manipulating abiotic factors (e.g., temperature, dissolved oxygen) and nutrition (as reviewed in Boglione et al. 2013b).

Linking a source to opercular deformities is difficult because multiple factors acting independently or synergistically, can cause deformities (Boglione and Costa 2011, Boglione et al. 2013a). These same factors can also produce other deformities in fishes. Known sources of deformities include nutritional deficiencies (e.g., vitamins A and C, fatty acids; Boglione et al. 2013b, Cahu et al. 2003, Fernandez et. al 2008, Gapasin et al. 1998, Gapasin and Duray 2001), poor water quality (e.g., temperature extremes, low dissolved oxygen; Georgakopoulou et al. 2010, Abdel et al. 2004, Boglione et al. 2013b), contaminants such as heavy metals (Lemly 1997a, 1997b, 2002; Hamilton 2005a, 2005b, 2005c, 2005d), and reduced genetic diversity, typically shown in hatchery stocks (Boglione et al. 2013b). These factors affect all life stages. Deformities can be the result of abiotic conditions experienced by females and transferred to offspring during oogenesis, the conditions eggs are exposed to once they are dispersed, or during larval development (Brown et al. 2010, Laaele and Lerner 1981, Lemly 1997a).

Potential teratogens in the San Juan River include cooler water temperatures and heavy metals. Cool water temperatures from hypolimnetic dam releases affect egg and larval development by altering developmental pathways (Georgakopoulou et al. 2010). Elevated levels of selenium are found in the sediments, flora, and fauna in the San Juan River (Thomas et al. 1998). This is attributed to leaching of naturally occuring selenium found in shale in the San Juan River watershed into irrigation returns to the river, and consequent bioaccumulation throughout the foodweb (Thomas et. al 1998). Deformities are produced in response to dietary exposure of parent fish and subsequent deposition of selenium in eggs (Hamilton 2005b, Lemly 1997a, 1997b). The presence of deformities in early life stages, in conjuction with high selenium levels in sediment, water, or biota, may be used as a bioindicator (Lemly 1997b). Problems from toxins may be compounded by low spring runoff and low summer flow that could concentrate toxins in low velocity habitats occupied by larval catostomids.

Although studies pertaining to histology and toxicology of age-0 San Juan River catostomids are outside the scope of this study, specimens were provided for preliminary analyses of future research questions posed by the SJRBRIP. As requested by the U.S. Bureau of Reclamation, in winter 2013 –

2014, specimens were forwarded to researchers for both histological and toxicological preliminary analyses. For histological analysis, Flannelmouth Sucker and Razorback Sucker specimens with severe opercular deformities (level 2) and no deformities (control; level 0) were sent to Dr. Wolfgang K. Vogelbein (Virginia Institute of Marine Science, College of William and Mary). Flannelmouth Sucker from one collection in July 2007 from Reach 5 comprised both the experimental (n = 6 fish with severely deformed opercles) and control (n = 5) specimens. Razorback Sucker with severe opercular deformities from Reach 1 (n = 6) and specimens lacking opercular deformities (n = 5) from Reach 3 were also submitted for analysis.

Sharon K. Taylor, D.V.M, Ph.D. (U.S. Bureau of Reclamation, Denver Federal Center) provided expertise and direction for preliminary toxicological analysis of age-0 San Juan River catostomids. An initial test for toxins would ideally measure metals, herbicides, and pesticides in fishes, depending on the amount of material (mass) available. As the minimum mass required for analysis would have significantly depleted available material (curated in Division of Fishes at MSB), the Razorback Suckers were not in included in the initial toxicological analysis. Instead, juvenile Flannelmouth Suckers with severe opercular deformities (n = 47; 14.15 g) were selected from river-wide July 2013 samples (Reaches 5 to 1) and sent to a private analytical environmental testing laboratory (TestAmerica, Pittsburgh) where the specimens were homogenized and tested for metals and herbicides in January 2014. There was not enough material available to test for pesticides. The results of these two pilot investigations (histology and toxicology) will be provided to the SJRBRIP by the U.S. Bureau of Reclamation when available.

Levels of deformities in hatchery stock Razorback Sucker are low (2.1%) compared to wild-spawned Razorback Sucker (23.6%). However, because most of the hatchery stock fish were late-stage larvae, a more appropriate comparison is to the similarly low frequency of deformities (1.5%) displayed by wild-spawned juvenile Razorback Sucker. Because of the absence of early-stage larvae used in the study, the low levels of opercular deformities in age-0 hatchery stock Razorback Sucker does not eliminate an underlying genetic cause for opercular deformities in wild age-0 Razorback Sucker. A future study is needed to document the frequency of deformities in hatchery-stock fish with systematic sampling throughout the early-life stage development. The results of a future study can determine if opercular deformities are prevalent in hatchery stock Razorback Sucker; however, an absence of deformities though all life stages will not preclude underlying genetic predisposition to deformities that may display when larvae are exposed to environmental conditions in the wild. Comparisons between wild and hatchery reared fish may not provide clear answers because multiple factors can contribute to deformity levels which are different in the wild than in a more controlled environment of a hatchery.

The ultimate recovery of Razorback Sucker depends on natural reproduction and recruitment. Larval fishes and early life stages in particular are already marked by high mortality rates, and deformities that impair or reduce survival likely contribute to their decline. Opercular deformities in age-0 Razorback Sucker may be a factor limiting recruitment to subadult and adult life stages. Continued monitoring of the larval fish community and documentation of potentially lethal opercular deformities is critical to timely identify future trends. Further, research specifically aimed to determine the causes of opercular deformities is warranted, as results might favorably lead to management actions that mitigate the incidence of deformities.

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LITERATURE CITED

Abdel, I., E. Abellan, O. Lopez-Albors, P. Valdes, M.J. Nortes, and A. Garcıa-Alcazar. 2004. Abnormalities in the juvenile stage of sea bass (*Dicentrarchus labrax* L.) reared at different temperatures: types, prevalence and effect on growth. Aquaculture International 12: 523–538.

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267-281 *in* B. N. Petrov and F. Csaki, editors. Second International Symposium on Information Theory. Akademiai, Budapest.

Al-Harbi, A. H. 2001. Skeletal deformities in cultured common carp *Cyprinus carpio* L. Asian Fisheries Science 14: 247-254.

Auer, N. A., (ed.). 1982. Identification of larval fishes of the Great Lakes basin with emphasis on the Lake Michigan drainage. Great Lakes Fishery Commission, Ann Arbor, MI 48105. Special Publication 82-3: 744 pp.

Barahona-Fernandes, M. H. 1982. Body deformation in hatchery reared European sea bass *Dicentrarchus labrax* (L.). Types, prevalence and effect of fish survival. Journal of Fish Biology 21: 239-249.

Beraldo P., M. Pinosa, E. Tibaldi, and B. Canavese. 2003. Abnormalities of the operculum in gilthead seabream (*Sparus aurata*): morphological description. Aquaculture 220: 89–99.

Beraldo P., and B. Canavese. 2011. Recovery of opercular anomalies in gilthead sea bream, *Sparus aurata* L.: morphological and morphometric analysis. Journal of Fish Diseases 34: 21–30.

Bezzerides, N., and K. R. Bestgen. 2002. Status review of roundtail chub *Gila robusta*, flannelmouth sucker *Catostomus latipinnis*, and bluehead sucker *Catostomus discobolus* in the Colorado River basin. Colorado State University Larval Fish Laboratory, Fort Collins, CO.

Bliesner, R., and V. Lamarra. 1999. San Juan River habitat studies, Annual Report. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM.

Boglione C., and C. Costa. 2011. Skeletal deformities and juvenile quality. Pages 233-294 *in* M. Pavlidis and C. Mylonas, editors. Sparidae: Biology and Aquaculture of Gilthead Sea Bream and Other Species. Wiley-Blackwell, Oxford, UK.

Boglione, C., E. Gisbert, P. Gavaia, P. E. Witten, M. Moren, S. Fontagne, and G. Koumoundouros. 2013a. Skeletal anomalies in reared European fish larvae and juveniles. Part 1: normal and anomalous skeletogenic processes. Reviews in Aquaculture 5: S99-S120.

Boglione, C., E. Gisbert, P. Gavaia, P. E. Witten, M. Moren, S. Fontagne, and G. Koumoundouros. 2013b. Skeletal anomalies in reared European fish larvae and juveniles. Part 2: main typologies, occurrences and causative factors. Reviews in Aquaculture 5: S121-S167.

Brandenburg, W. H. 2000. Razorback sucker larval fish survey in the San Juan River during 1998 and 1999. Annual report. San Juan River Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM.

Brown, C. L., D. M. Power, and J. M. Nunez. 2010. Disorders of Development in Fish. Pages 166-181 *in* J. F. Leatherland and P. T. K. Woo, editors. Fish Diseases and Disorders: Non-infectious Disorders. CABI Publishing, Wallingford, UK.

Burnham, K.P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. Springer-Verlag, New York.

Cahu C. L., J. L. Zambonino Infante, and V. Barbosa. 2003. Phospholipid level in dietary lipid fraction is determining for sea bass (*Dicentrarchus labrax*) larval development. British Journal of Nutrition 90: 21–28.

Carpenter, J., and G. A. Mueller. 2008. Small non-native fishes as predators of larval razorback suckers. The Southwestern Naturalist 53: 236-242.

Colorado River Fish and Wildlife Council. 2004. Range-wide conservation agreement for roundtail chub *Gila robusta*, bluehead sucker *Catostomus discobolus*, and flannelmouth sucker *Catostomus latipinnis*. Utah Department of Natural Resources, Salt Lake City, UT.

Cook, S. J., C. M. Blunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservations strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121: 317-331.

Douglas, M. E., and P. C. Marsh. 1998. Population and survival estimates of *Catostomus latipinnis* in Northern Grand Canyon, with distribution and abundance of hybrids with *Xyrauchen texanus*. Copeia 1998: 915-925.

Dowling, T., M. Saltzgiver, and P. Marsh. 2012. Genetic structure within and among populations of the endangered razorback sucker (*Xyrauchen texanus*) as determined by analysis of microsatellites. Conservation Genetics 13: 1073-1083.

Farrington, M. A., W. H. Brandenburg, and S. P. Platania. 2013. Colorado pikeminnow and razorback sucker larval fish survey in the San Juan River during 2012, annual report. San Juan River Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. http://www.fws.gov/southwest/sjrip/pdf/DOC_Colorado_pikeminnow_razorback_sucker_larval_fish_surveys_San_Juan_River-2012.pdf. (March 2014).

Fernandez I, F. Hontoria, J. B. Ortiz-Delgado, Y. Kotzamanis, A. Estevez, and J. L. Zambonino-Infante. 2008. Larval performance and skeletal deformities in farmed gilthead sea bream (*Sparus aurata*) fed with graded levels of vitamin A enriched rotifers (*Brachionus plicatilis*). Aquaculture 283: 102–115.

Fraser, M. R., and R. de Nys. 2005. The morphology and occurrence of jaw and operculum deformities in cultured barramundi (*Lates calcarifer*) larvae. Aquaculture 250: 496-503.

Furr, D. W. 2013. San Juan River Razorback Sucker (*Xyrauchen texanus*) population augmentation: 2012 annual report. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. Available: http://www.fws.gov/southwest/sjrip/pdf/DOC_Augmentation_San_Juan_River_razorback_sucker_population_2012.pdf. (March 2014).

Galeotti M., P. Beraldo, S. de Dominis, L. D'Angelo, R. Ballestrazzi, and R. Musetti. 2000. A preliminary histological and ultra-structural study of opercular anomalies in gilthead sea bream larvae (*Sparus aurata*). Fish Physiology and Biochemistry 22: 151–157.

Gapasin, R. S. J., R. Bombeo, P. Lavens, P. Sorgeloos, and H. Nelis. 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish *Chanos chanos* larval performance. Aquaculture 162: 269–286.

Gapasin, R. S. J., and M. N. Duray. 2001. Effects of DHA-enriched live food on growth, survival and incidence of opercular deformities in milkfish (*Chanos chanos*). Aquaculture 193: 49–63.

Georgeakopoulou E., P. Katharios, P. Divanach, and G. Koumoundouros. 2010. Effect of temperature on the development of skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758). Aquaculture 208: 13-19.

Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005a. Selenium impacts on razorback sucker, Colorado: Colorado River. I. Adults. Ecotoxicology and Environmental Safety 61: 7-31.

Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005b. Selenium impacts on razorback sucker, Colorado: Colorado River. II. Eggs. Ecotoxicology and Environmental Safety 61: 32-43.

Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005c. Selenium impacts on razorback sucker, Colorado: Colorado River. III. Larvae. Ecotoxicology and Environmental Safety 61: 168-189.

Hamilton, S. J., K. M. Holley, K. J. Buhl, and F. A. Bullard. 2005d. Reduced growth and survival of larval razorback sucker fed selenium-laden zooplankton. Ecotoxicology and Environmental Safety 61: 190-208.

Harvey, B. C. 1991. Interaction of abiotic and biotic factors influences larval fish survival in an Oklahoma stream. Canadian Journal of Fisheries and Aquatic Science 48: 1476-1480.

Holden, P. B. (Ed.). 1999. Flow recommendations for the San Juan River. San Juan River Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM.

Jennings, M. J., and D. P. Philipp. 1994. Biotic and abiotic factors affecting survival of early life history intervals of stream-dwelling sunfish. Environmental Biology of Fishes 39:153-159.

JMP®, Version 7.0. SAS Institute Inc., Cary, NC, 1989-2007.

Kause A., O. Ritola, and T. Paananen. 2007. Changes in the expression of genetic characteristics across cohorts in skeletal deformations of farmed salmonids. Genetics Selection Evolution 39: 529–543.

Koumoundouros, G. 2010. Morpho-anatomical abnormalities in Mediterranean marine aquaculture. Pages 125-148 *in*: G. Koumoundouros, editor. Recent Advances in Aquaculture Research. Transworld Research Network, Kerala, India.

Koumoundouros, G., G. Oran, P. Divanach, S. Stefanakis, and M. Kenouri. 1997. The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparition and description. Aquaculture 156: 165-177.

Laaele, H. W., and W. Lerner. 1981. Teratology and eary fish development. American Zoologist 21: 517-533.

Lashmett, K. 1993. Young-of-the-year fish survey of the lower San Juan River 1993. Unpublished report San Juan River Basin Recovery Implementation Program. Bureau of Reclamation, Durango, CO. 82 pp.

Lemly, A. D. 1997a. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety 37: 259-266.

Lemly A. D. 1997b. Environmental hazard of selenium in the Animas La Plata Water Development Project. Ecotoxicology and Environmental Safety 37(1): 92-96.

Lemly A. D. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57: 39-49.

Lindesjoo E., J. Thulin, B. Bengtsson, and U. Tjarnlund. 1994. Abnormalities of a gill cover bone, the operculum, in perch *Perca fluviatilis* from a pulp mill effluent area. Aquatic Toxicology 28: 189-207.

Miller, B. S., and A. W. Kendall. 2009. Early Life History Of Marine Fishes. University of California Press, Oakland, CA.

Minckley, W. L., P. C. Marsh, J. E. Deacon, T. E. Dowling, P. W. Hedrick, W. J. Matthews, and G. Mueller. 2003. A conservation plan for native fishes of the lower Colorado River. Bioscience 53: 219-232.

Patten, B. G. 1968. Abnormal freshwater fishes in Washington streams. Copeia 1968(2): 399-401.

Paperna, I., B. Ross, A. Colorni, and B. Colorni. 1980. Disease of marine fish cultured in Eliat mariculture project. Studies and Reviews. General Fisheries Council for the Mediterranean 57: 29– 32.

Platania, S. P. 1990. San Juan River ichthyofaunal study New Mexico-Utah 1987-1989, Final Report. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM.

Plunkett, S. R., and E. Snyder-Conn. 2000. Anomalies of larval and juvenile shortnose and lost river suckers in Upper Klamath Lake, Oregon. Klamath Falls Office, U.S. Fish and Wildlife Service, Klamath Falls, OR. Avaiable: http://ecos.fws.gov/ServCatFiles/reference/holding/21480?accessType=DOWNLOAD . (March 2014).

Ptacek, J. A., D. E. Rees, and W. J. Miller. 2005. Bluehead Sucker (*Catostomus discobolus*): a technical conservation assessment. [Online]. U.S. Department of Agriculture Forest Service, Rocky Mountain Region. Available: http://www.fs.fed.us/r2/projects/scp/assessments/blueheadsucker.pdf (March 2014).

Rees, D. E., J. A. Ptacek, R. J. Carr, and W. J. Miller. 2005. Flannelmouth Sucker (*Catostomus latipinnis*): a technical conservation assessment. U.S. Department of Agriculture Forest Service, Rocky Mountain Region. Available: http://www.fs.fed.us/r2/projects/scp/assessments/flannelmouthsucker.pdf. (March 2014).

Ryden, D. W., and F. K. Pfeifer. 1994. An experimental stocking plan for razorback sucker in the San Juan River. U.S. Fish and Wildlife Service, Grand Junction, CO.

Ryden, D. W. 1997. Five-year augmentation plan for razorback sucker in the San Juan River. U.S. Fish and Wildlife Service, Grand Junction, CO.

San Juan River Basin Recovery Implementation Program. 1995. Program Document. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. Available: http://www.fws.gov/southwest/sjrip/pdf/DOC_Program_Document_1995.pdf. (March 2014).

San Juan River Basin Recovery Implementation Program. 2013. Long-range plan, in draft. San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. Available: http://www.fws.gov/southwest/sjrip/pdf/DOC_2013_SJRRIP_Long_Range_Plan.pdf. (March 2014).

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Schleicher, B. J., and D. W. Ryder. 2013. Long term monitoring of sub-adult and adult large-bodied fishes in the San Juan River: 2013 interim progress report. San Juan River Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. Available: http://www.fws.gov/southwest/sjrip/pdf/DOC_Long_term_monitoring_sub-adult_adult_large-bodied_fishes_San_Juan_River_2013.pdf. (February 2015).

Schleicher, B. J., and D. W. Ryder. 2012. Long term monitoring of sub-adult and adult large-bodied fishes in the San Juan River: 2012 interim progress report. San Juan River Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM. Available: http://www.fws.gov/southwest/sjrip/pdf/DOC_Long_term_monitoring_sub-adult_adult_large-bodied_fishes_San_Juan_River_2012.pdf. (March 2014).

Snyder, D. E., 1981. Contributions to a guide to the cypriniform fish larvae of the Upper Colorado River system in Colorado. U.S. Bureau of Land Management, Biological Sciences Series 3, Denver, CO. 81 pp.

Snyder, D. E., and R. T. Muth. 2004. Catostomid fish larvae and early juveniles of the Upper Colorado river basin-morphological descriptions, comparisons, and computer-interactive key. Technical publication No. 42. Colorado Division of Wildlife. Larval Fish Laboratory, Colorado State University, Fort Collins, CO.

Sun, P. L., W. E. Hawkins, R. M. Overstreet, and N. J. Brown-Peterson. 2009. Morphological deformities as biomarkers in fish from contaminated rivers in Taiwan. International Journal of Environmental Research and Public Health 6: 2307-2331.

Tave D., and T. S. Handwerker. 1994. Semi-operculum: A non-heritable birth defect in *Tilapia nilotica*. Journal of the World Aquaculture Society 25: 333-336.

Thomas, C. L, R. M. Wilson, J. D. Lusk, R. S. Bristol, and A. R. Shineman. 1998. Detailed study of selenium and selected constituents in water, bottom sediment, soil, and biota associated with irrigation drainage in the San Jan River are, New Mexico, 1991-95. USGS Water-Resources Investigations Report 98-4213. Available: http://www.fws.gov/southwest/sjrip/DR_WQC.cfm. (February 2014).

Tyus, H. M., and J. F. Saunders. 2000. Nonnative fish control and endangered fish recovery: lessons from the Colorado River. Fisheries 25: 17-24.

Tyus, H. M., and C. A. Karp. 1990. Spawning and movements of razorback sucker, *Xyrauchen texanus*, in the Green River Basin of Colorado and Utah. The Southwestern Naturalist 35: 427-433.

U.S. Fish and Wildlife Service. 1991. Endangered and threatened wildlife and plants; the razorback sucker (*Xyrauchen texanus*) determined to be an endangered species. Federal Register 59: 54957-54967.

U.S. Fish and Wildlife Service. 1998. Razorback sucker recovery plan. U.S. Fish and Wildlife Service, Region 6, Denver, CO.

Verhaegen Y., D. Adriaens, T. de Wolf, P. Dhert, and P. Sorgeloos. 2007. Deformities in larval gilthead sea bream (*Sparus aurata*): a qualitative and quantitative analysis using geometric morphometrics. Aquaculture 268: 156–168.

Winemiller K. O., and D. H. Tayler. 1982. Inbreeding depression in the convict cichlid, *Cichlasoma nigrofasciatum* (Baird and Girard). Journal of Fish Biology 21: 399-402.

Appendix I. Accounting, by years, of the number of larval and juvenile age-0 San Juan River Bluehead Sucker examined for this project and the annual frequency of occurrence of opercular deformities.

Year	# fish preserved	# fish examined for deformities	% of fish collected examined for deformities	# fish with deformities	% of examined fish with deformities
1998	1,810	173	9.6	22	12.7
1999	379	0	0.0	- - -	- - -
2000	4,668	173	3.7	0	0.0
2001	528	0	0.0	- - -	- - -
2002	3,816	708	18.6	10	1.4
2003	1,359	486	35.8	24	4.9
2004	6,800	509	7.5	50	9.8
2005	7,558	0	0.0	- - -	- - -
2006	4,060	313	7.7	6	1.9
2007	7,996	325	4.1	32	9.8
2008	1,418	371	26.2	21	5.7
2009	1,165	320	27.5	43	13.4
2010	2,410	504	20.9	45	8.9
2011	4,502	717	15.9	51	7.1
2012	7,929	3,966	50.0	237	6.0
TOTAL	56,398	8,565	15.5	541	6.3

Appendix II. Accounting, by years, of the number of larval and juvenile age-0 San Juan River Flannelmouth Sucker examined for this project and the annual frequency of occurrence of opercular deformities.

Year	# fish preserved	# fish examined for deformities	% of fish collected examined for deformities	# fish with deformities	% of examined fish with deformities
1998	11,108	2,216	19.9	60	2.7
1999	7,340	1,026	14.0	24	2.3
2000	11,154	6,194	55.5	133	2.1
2001	7,636	2,434	31.9	88	3.6
2002	7,706	5,030	65.3	38	0.8
2003	5,143	3,258	63.3	180	5.5
2004	3,142	1,050	33.4	40	3.8
2005	3,115	1,424	45.7	80	5.6
2006	5,360	3,613	67.4	26	0.7
2007	16,507	3,537	21.4	408	11.5
2008	20,051	5,264	26.3	92	1.7
2009	3,336	932	27.9	25	2.7
2010	5,294	1,590	30.0	41	2.6
2011	5,851	2,744	46.9	105	3.8
2012	6,757	5,104	75.5	634	12.4
TOTAL	119,500	45,416	38.0	1,974	4.3

Appendix III. Accounting, by years, of the number of larval and juvenile age-0 San Juan River Razorback Sucker examined for this project and the annual frequency of occurrence of opercular deformities.

Year	# fish preserved	# fish examined for deformities	% of fish collected examined for deformities	# fish with deformities	% of examined fish with deformities
1998	2	0	0	- - -	- - -
1999	7	2	28.6	0	0.0
2000	129	30	23.3	16	53.3
2001	50	28	56.0	7	25.0
2002	818	403	49.3	54	13.4
2003	450	169	37.6	29	17.2
2004	41	2	4.9	1	50.0
2005	19	6	31.6	1	16.7
2006	202	66	32.7	9	13.6
2007	203	66	32.5	12	18.2
2008	126	6	4.8	6	100.0
2009	272	117	43.0	24	20.5
2010	1,250	107	8.6	43	40.2
2011	1,065	146	13.7	44	30.1
2012	1,778	256	14.4	85	33.2
TOTAL	6,412	1,404	21.9	331	23.6

Appendix IV. Razorback Sucker during the three larval developmental stages (A, B, and C) and as a juvenile (D). Information on opercular development, age, and length range of Razorback Sucker during these four developmental stages are provided.

Developmental Stage:	Protolarvae
Operculum Formed:	No
Age in Days:	7 – 10 days
Total Length:	9 – 12 mm TL

Developmental Stage:	Mesolarvae
Operculum Formed:	Fully formed by 15 mm TL
Age in Days:	10 – 30 days
Total Length:	11 – 20 mm TL

Developmental Stage:	Metalarvae
Operculum Formed:	Yes
Age in Days:	30 – 50 days
Total Length:	18 – 30 mm TL

Developmental Stage:	Juvenile
Operculum Formed:	Yes
Age in Days:	> 50 days
Total Length:	> 27 mm TL

Appendix V. A 12.8 mm TL flexion mesolarval Razorback Sucker above a U.S. penny to provide a size reference.